



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12N 15/00, C07K 7/10, 13/00 A61K 37/02, 27/00		A1	(11) International Publication Number: WO 92/07073 (43) International Publication Date: 30 April 1992 (30.04.92)		
(21) International Application Number: PCT/US91/07635 (22) International Filing Date: 18 October 1991 (18.10.91)		(74) Agent: PITCHER, Edmund, R.; Testa, Hurwitz & Thibault, Exchange Place, 53 State Street, Boston, MA 02109-2809 (US).			
(30) Priority data: 599,543 18 October 1990 (18.10.90) US		(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).			
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(54) Title: OSTEOGENIC PEPTIDES					
(57) Abstract					
<p>Disclosed are 1) the cDNA and amino acid sequences for novel polypeptide chains useful as subunits of dimeric osteogenic proteins, 2) osteogenic devices comprising these proteins in association with an appropriate carrier matrix, 3) methods of producing the polypeptide chains using recombinant DNA technology, and 4) methods of using the osteogenic devices to mimic the natural course of endochondral bone formation in mammals.</p>					

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Osteogenic peptides

Background of the Invention

5 This invention relates to novel polypeptide chains and to osteogenic proteins comprising these polypeptide chains which are capable of inducing osteogenesis in mammals; to genes encoding the polypeptide chains; to methods for their production using recombinant DNA
10 techniques, and to bone and cartilage repair procedures using the osteogenic proteins.

Mammalian bone tissue is known to contain one or more proteinaceous materials, presumably active during
15 growth and natural bone healing, which can induce a developmental cascade of cellular events resulting in endochondral bone formation. This active factor (or factors) has variously been referred to in the literature as bone morphogenetic or morphogenic
20 protein, bone inductive protein, osteogenic protein, osteogenin, or osteoinductive protein.

The developmental cascade of bone differentiation consists of recruitment of mesenchymal cells,
25 proliferation of progenitor cells, calcification of cartilage, vascular invasion, bone formation, remodeling, and finally marrow differentiation (Reddi (1981) Collagen Rel. Res. 1:209-226).

30 Though the precise mechanisms underlying these phenotypic transformations are unclear, it has been shown that the natural endochondral bone differentiation activity of bone matrix can be dissociatively extracted and reconstituted with

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inactive residual collagenous matrix to restore full bone induction activity (Sampath and Reddi, (1981) Proc. Natl. Acad. Sci. USA 78:7599-7603). This provides an experimental method for assaying protein
05 extracts for their ability to induce endochondral bone in vivo. Several species of mammals produce closely related protein as demonstrated by cross species implant experiments (Sampath and Reddi (1983) Proc. Natl. Acad. Sci. USA 80:6591-6595).

10 The potential utility of these proteins has been recognized widely. It is contemplated that the availability of the protein would revolutionize orthopedic medicine, certain types of plastic surgery, and various periodontal and craniofacial reconstructive
15 procedures.

The observed properties of these protein fractions have induced an intense research effort in various laboratories directed to isolating and identifying the pure factor or factors responsible for osteogenic
20 activity. The current state of the art of purification of osteogenic protein from mammalian bone is disclosed by Sampath et al. ((1987) Proc. Natl. Acad. Sci. USA 84: 7109-7113). Urist et al. (1984) Proc. Soc. Exp. Biol. Med. 173: 194-199 disclose a human osteogenic
25 protein fraction which was extracted from demineralized cortical bone by means of a calcium chloride-urea inorganic-organic solvent mixture, and retrieved by differential precipitation in guanidine-hydrochloride and preparative gel electrophoresis. The authors
30 report that the protein fraction has an amino acid composition of an acidic polypeptide and a molecular weight in a range of 17-18 kD.

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Urist et al. (1984) Proc. Natl. Acad. Sci. USA 81:
371-375 disclose a bovine bone morphogenetic protein
extract having the properties of an acidic polypeptide
and a molecular weight of approximately 18 kD. The
authors reported that the protein was present in a
fraction separated by hydroxyapatite chromatography,
and that it induced bone formation in mouse hindquarter
muscle and bone regeneration in trephine defects in rat
and dog skulls. Their method of obtaining the extract
from bone results in ill-defined and impure
preparations.

European Patent Application Serial No. 148,155,
published October 7, 1985, purports to disclose
osteogenic proteins derived from bovine, porcine, and
human origin. One of the proteins, designated by the
inventors as a P3 protein having a molecular weight of
22-24 kD, is said to have been purified to an
essentially homogeneous state. This material is
reported to induce bone formation when implanted into
animals.

International Application No. PCT/087/01537,
published January 14, 1988, discloses an impure
fraction from bovine bone which has bone induction
qualities. The named applicants also disclose putative
"bone inductive factors" produced by recombinant DNA
techniques. Four DNA sequences were retrieved from
human or bovine genomic or cDNA libraries and expressed
in recombinant host cells. While the applicants stated
that the expressed proteins may be bone morphogenic
proteins, bone induction was not demonstrated,
suggesting that the recombinant proteins are not
osteogenic. The same group reported subsequently
(Science, 242:1528, Dec, 1988) that three of the four

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factors induce cartilage formation, and postulate that bone formation activity "is due to a mixture of regulatory molecules" and that "bone formation is most likely controlled ... by the interaction of these 05 molecules." Again, no bone induction was attributed to the products of expression of the cDNAs. See also Urist et al., EP0,212,474 entitled Bone Morphogenic Agents.

Wang et al. (1988) Proc. Nat. Acad. Sci. USA 85: 10 9484-9488 discloses the purification of a bovine bone morphogenetic protein from guanidine extracts of demineralized bone having cartilage and bone formation activity as a basic protein corresponding to a molecular weight of 30 kD determined from gel elution. 15 Purification of the protein yielded proteins of 30, 18 and 16 kD which, upon separation, were inactive. In view of this result, the authors acknowledged that the exact identity of the active material had not been determined.

20 Wang et al. (1990) Proc. Nat. Acad. Sci. USA 87: 2220-2227 describes the expression and partial purification of one of the cDNA sequences described in PCT 87/01537. Consistent cartilage and/or bone formation with their protein requires a minimum of 600 25 ng of 50% pure material.

International Application No. PCT/89/04458 published April 19, 1990 (Int. Pub. No. WO90/003733), describes the purification and analysis of a family of osteogenic factors called "P3 OF 31-34". The protein 30 family contains at least four proteins, which are characterized by peptide fragment sequences. The impure mixture P3 OF 31-34 is assayed for osteogenic

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05 activity. The activity of the individual proteins is neither assessed nor discussed.

It is an object of this invention to provide novel polypeptide chains useful as subunits of dimeric osteogenic proteins capable of endochondral bone formation in allogenic and xenogenic implants in mammals, including humans. Another object is to provide genes encoding these polypeptide chains and methods for the production of osteogenic proteins comprising these polypeptide chains using recombinant DNA techniques, as well as to provide antibodies capable of binding specifically to these proteins.

These and other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

Summary of the Invention

This invention provides novel polypeptide chains useful as either one or both subunits of dimeric osteogenic proteins which, when implanted in a 05 mammalian body in association with a matrix, can induce at the locus of the implant the full developmental cascade of endochondral bone formation and bone marrow differentiation.

A key to these developments was the elucidation of 10 amino acid sequence and structure data of native bovine osteogenic protein. A protocol was developed which results in retrieval of active, substantially pure osteogenic protein from bovine bone having a half-maximum bone forming activity of about 0.8 to 1.0 ng 15 per mg of implant. The availability of the material enabled the inventors to elucidate key structural details of the protein necessary to achieve bone formation. Knowledge of the protein's amino acid sequence and other structural features enabled the 20 identification and cloning of native genes in the human genome.

Consensus DNA sequences based on partial sequence data and observed homologies with regulatory proteins disclosed in the literature were used as probes for 25 extracting genes encoding osteogenic protein from human genomic and cDNA libraries. One of the consensus sequences was used to isolate a previously unidentified gene which, when expressed, encoded a protein comprising a region capable of inducing endochondral 30 bone formation when properly modified, incorporated in a suitable matrix, and implanted as disclosed herein. The gene, called "hOP1" or "OP-1" (human OP-1), is

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described in greater detail in copending U.S. 422,699, the disclosure of which is herein incorporated by reference.

In its native form, hOP1 expression yields an immature translation product ("hOP1-PP", where "PP" refers to "prepro form") of about 400 amino acids that subsequently is processed to yield a mature sequence of 139 amino acids ("OP1-18"). The active region (functional domain) of the protein comprises the C-terminal 97 amino acids of the hOP1 sequence ("OPS"). A long active sequence is OP7 (comprising the C-terminal 102 amino acids).

Further probing of mammalian cDNA libraries (human and mouse) with sequences specific to hOP1 also has identified novel OP1-like sequences herein referred to as "OP2" ("hOP2" or "mOP2"). The OP2 proteins share significant amino acid sequence homology, approximately 74%, with the active region of the OP1 proteins (e.g., OP7), and less homology with the intact mature form (e.g., OP1-18, 58% amino acid homology).

The amino acid sequence of the osteogenic proteins disclosed herein also share significant homology with various of the regulatory proteins on which the consensus probe was modeled. In particular, the proteins share significant homology in their C-terminal sequences, which comprise the active region of the osteogenic proteins. (Compare, for example, OP7 with DPP from Drosophila and Vgl from Xenopus. See, for example, U.S. Pat. No. 5,011,691). In addition, these proteins share a conserved six or seven cysteine skeleton in this region (e.g., the linear arrangement of these C-terminal cysteine residues is conserved in

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the different proteins.) See, for example, OP7, whose sequence defines the seven cysteine skeleton, or OPS, whose sequence defines the six cysteine skeleton. The OP2 proteins also contain an additional cysteine 05 residue within this region.

Thus, in one preferred aspect, the invention comprises osteogenic proteins comprising a polypeptide chain comprising an amino acid sequence described by Seq. ID No. 3 or 5, including allelic and species 10 variants thereof, and naturally-occurring or biosynthetic mutants, such that a dimeric protein comprising this polypeptide chain has a conformation capable of inducing endochondral bone formation when implanted in a mammal in association with a suitable 15 matrix. Useful proteins include the full-length protein, mature proteins and truncated proteins comprising the functional domain described by the C-terminal.

In addition, the invention is not limited to these 20 specific constructs. Thus, the osteogenic proteins of this invention comprising any of these polypeptide chains may include forms having varying glycosylation patterns, varying N-termini, a family of related 25 proteins having regions of amino acid sequence homology which may be naturally occurring or biosynthetically derived, and active truncated or mutated forms of the native amino acid sequence, produced by expression of recombinant DNA in prokaryotic or eukaryotic host cells. Active sequences useful as osteogenic proteins 30 of this invention are envisioned to include proteins capable of inducing endochondral bone formation when implanted in a mammal in association with a matrix and having at least a 70% sequence homology, preferably at

least 80%, with the amino acid sequence of OPS. This includes longer forms of a given protein, as well as allelic variants and muteins, including addition and deletion mutants, such as those which may alter the 05 conserved C-terminal cysteine skeleton, provided that the alteration still allows the protein to form a dimeric species having a conformation capable of inducing bone formation in a mammal when implanted in the mammal in association with a matrix.

10 The novel polypeptide chains and the osteogenic proteins they comprise can be expressed from intact or truncated cDNA or from synthetic DNAs in prokaryotic or eucaryotic host cells, and then purified, cleaved, refolded, dimerized, and implanted in experimental 15 animals. Currently preferred host cells include E.coli or mammalian cells, such as CHO, COS or BSC cells. The osteogenic protein of the invention may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of 20 amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

Thus, in view of this disclosure, skilled genetic 25 engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both prokaryotes 30 and eucaryotes, to produce large quantities of active proteins capable of inducing bone formation in mammals including humans. In view of this disclosure, those skilled in the art, using standard immunology

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techniques also may create antibodies capable of binding specifically to the osteogenic proteins disclosed herein, including fragments thereof.

The osteogenic proteins are useful in clinical applications in conjunction with a suitable delivery or support system (matrix). The matrix is made up of particles of porous materials. The pores must be of a dimension to permit progenitor cell migration and subsequent differentiation and proliferation. The particle size should be within the range of 70 - 850 mm, preferably 150mm - 420mm. It may be fabricated by close packing particulate material into a shape spanning the bone defect, or by otherwise structuring as desired a material that is biocompatible (non-inflammatory) and, biodegradable in vivo to serve as a "temporary scaffold" and substratum for recruitment of migratory progenitor cells, and as a base for their subsequent anchoring and proliferation. Currently preferred carriers include particulate, demineralized, guanidine extracted, species-specific (allogenic) bone, and specially treated particulate, protein extracted, demineralized, xenogenic bone. Optionally, such xenogenic bone powder matrices also may be treated with proteases such as trypsin and/or fibril modifying agents to increase the intraparticle intrusion volume and surface area. Useful agents include solvents such as dichloromethane, trichloroacetic acid, acetonitrile and acids such as trifluoroacetic acid and hydrogen fluoride. Alternatively, the matrix may be treated with a hot aqueous medium having a temperature within the range of about 37°C to 75°C, including a heated acidic aqueous medium. Other potentially useful matrix materials comprise collagen, homopolymers and copolymers of glycolic acid and lactic acid,

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hydroxyapatite, tricalcium phosphate and other calcium phosphates.

The osteogenic proteins and implantable osteogenic devices enabled and disclosed herein will permit the physician to obtain optimal predictable bone formation to correct, for example, acquired and congenital craniofacial and other skeletal or dental anomalies (Glowacki et al. (1981) Lancet 1:959-963). The devices may be used to induce local endochondral bone formation in non-union fractures as demonstrated in animal tests, and in other clinical applications including dental and periodontal applications where bone formation is required. Another potential clinical application is in cartilage repair, for example, in the treatment of osteoarthritis.

Brief Description of the Drawing

The foregoing and other objects of this invention, the various features thereof, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings, in which:

FIGURE 1 compares the amino acid sequences of the mature mOP-2 and hOP-2 polypeptide chains: hOP2-A and mOP2-A; and

FIGURE 2 compares the amino acid sequences of the mature OP1 and OP2 polypeptide chains: OP1-18, mOP1-S, hOP2-A and mOP2-A.

Description

Purification protocols first were developed which enabled isolation of the osteogenic protein present in crude protein extracts from mammalian bone. (See PCT WO 89/09787, published 19-OCT-89, and U.S. Serial No. 05 179,406 filed April 8, 1988, now U.S. Patent No. 4,968,950). The development of the procedure, coupled with the availability of fresh calf bone, enabled isolation of substantially pure bovine osteogenic protein (bOP). bOP was characterized significantly; 10 its ability to induce cartilage and ultimately endochondral bone growth in cat, rabbit, and rat were demonstrated and studied; it was shown to be able to induce the full developmental cascade of bone formation previously ascribed to unknown protein or proteins in 15 heterogeneous bone extracts. This dose dependent and highly specific activity was present whether or not the protein was glycosylated (see Sampath et al., (1990) J. Biol. Chem. 265: 13198-13205). Sequence data obtained from the bovine materials suggested probe designs which 20 were used to isolate human genes. The OP human counterpart proteins have now been expressed and extensively characterized.

These discoveries enabled preparation of DNAs encoding totally novel, non-native protein constructs 25 which individually as homodimers and combined with other species as heterodimers are capable of producing true endochondral bone (see PCT WO 09788, published 19-OCT-89, and US Serial No. 315,342, filed 23-FEB-89, now U.S. Patent No. 5,011,691). They also permitted 30 expression of the natural material, truncated forms, muteins, analogs, fusion proteins, and various other variants and constructs, from cDNAs and genomic DNAs retrieved from natural sources or from synthetic DNA produced using the techniques disclosed herein and

using automated, commercially available equipment. The DNAs may be expressed using well established molecular biology and recombinant DNA techniques in prokaryotic or eucaryotic host cells, and may be oxidized and 05 refolded in vitro if necessary, to produce biologically active protein.

One of the DNA sequences isolated from human genomic and cDNA libraries encoded a previously unidentified gene, referred to herein as OP1. The 10 protein encoded by the isolated DNA was identified originally by amino acid homology with proteins in the TGF- β family. Consensus splice signals were found where amino acid homologies ended, designating exon-intron boundaries. Three exons were combined to obtain 15 a functional TGF- β -like domain containing seven cysteines. (See, for example, U.S. Patent No. 5,011,691, or Ozkaynak, E. et al., (1990) EMBO. 9: 2085-2093).

The full-length cDNA sequence for hOP1, and its 20 encoded "prepro" form "hOP1-PP," which includes an N-terminal signal peptide sequence, are disclosed in Seq. ID No. 1 (residues 1-431). The mature form of the hOP1 protein expressed in mammalian cells, "OP1-18", is described by amino acid residues 293-431 of Seq. ID 25 No. 1. The full length form of hOP1, as well as various truncated forms of the gene, and fused genes, have been expressed in E. coli and numerous mammalian cells (see, for example, published PCT application WO 91/05802, published 2-MAY-91) and all have been shown 30 to have osteogenic activity when implanted in a mammal in association with a suitable matrix.

Given the foregoing amino acid and DNA sequence information, various nucleic acids (RNAs and DNAs) can be constructed which encode at least the active region of the hOP1 protein (e.g., OPS or OP7) and various analogs thereof (including allelic and species variants and those containing genetically engineered mutations), as well as fusion proteins, truncated forms of the mature proteins, and similar constructs. Moreover, DNA hybridization probes can be constructed from fragments 10 of the hOP1 DNA or designed de novo based on the hOP1 DNA or amino acid sequence. These probes then can be used to screen different genomic and cDNA libraries to identify additional osteogenic proteins.

The DNAs can be produced by those skilled in the art using well known DNA manipulation techniques involving genomic and cDNA isolation, construction of synthetic DNA from synthesized oligonucleotides, and cassette mutagenesis techniques. 15-100mer oligonucleotides may be synthesized on a Biosearch DNA 20 Model 8600 Synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer. The DNA then may be electroeluted from the gel. Overlapping oligomers may be phosphorylated by T4 25 polynucleotide kinase and ligated into larger blocks which may also be purified by PAGE.

DNAs used as hybridization probes may be labelled (e.g., as with a radioisotope, by nick-translation) and used to identify clones in a given library containing DNA to which the probe hybridizes, following techniques 30 well known in the art. The libraries may be obtained commercially or they may be constructed de novo using conventional molecular biology techniques. Further information on DNA library construction and

hybridization techniques can be found in numerous texts known to those skilled in the art. See, for example, F.M. Ausubel., ed., Current Protocols in Molecular Biology-Vol. 1, (1989). In particular, see unit 5,

05 "Construction of Recombinant DNA Libraries" and Unit 6, "Screening of Recombinant Libraries."

The DNA from appropriately identified clones then can be isolated, subcloned (preferably into an expression vector), and sequenced. Plasmids containing 10 sequences of interest then can be transfected into an appropriate host cell for protein expression and further characterization. The host may be a prokaryotic or eucaryotic cell since the former's inability to glycosylate protein will not destroy the 15 protein's osteogenic activity. Useful host cells include E. coli, Saccharomyces, the insect/baculovirus cell system, myeloma cells, and various mammalian cells. The vector additionally may encode various sequences to promote correct expression of the 20 recombinant protein, including transcription promoter and termination sequences, enhancer sequences, preferred ribosome binding site sequences, preferred mRNA leader sequences, preferred signal sequences for protein secretion, and the like. The DNA sequence 25 encoding the gene of interest also may be manipulated to remove potentially inhibiting sequences or to minimize unwanted secondary structure formation. The recombinant osteogenic protein also may be expressed as a fusion protein. After being translated, the protein 30 may be purified from the cells themselves or recovered from the culture medium. All biologically active protein forms comprise dimeric species joined by disulfide bonds or otherwise associated, produced by oxidizing and refolding one or more of the various

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recombinant polypeptide chains within an appropriate eucaryotic cell or in vitro after expression of individual subunits. A detailed description of osteogenic protein expressed from recombinant DNA in E. 05 coli is disclosed in U.S. Serial No. 660,162, filed 27-FEB-91, the disclosure of which incorporated by reference herein. A detailed description of osteogenic protein expressed from recombinant DNA in numerous different mammalian cells is disclosed in PCT 10 WO91/05802, also incorporated herein by reference.

Finally, in view of the disclosure made herein, and using standard methodologies known in the art, persons skilled in the art can raise polyclonal and monoclonal antibodies against all or part of a polypeptide chain 15 disclosed herein, such that the antibodies are capable of binding specifically to an epitope on the polypeptide chain. Useful protocols can be found in, for example, Molecular Cloning-A Laboratory Manual (Sambrook et al. eds., Cold Spring Harbor Press 2nd ed. 20 1989). See Book 3, Section 18.

Exemplification

In an effort to identify additional DNA sequences encoding osteogenic proteins, a hybridization probe specific to the C-terminus of the DNA of mature OP-1 25 was prepared using a StuI-EcoRI digest fragment of OP-1 (base pairs 1034-1354 in Sequence ID No. 1), and labelled with ³²P by nick translation, as described in the art. As disclosed supra, the OP1 C-terminus encodes a key functional domain, e.g., the "active 30 region" for osteogenic activity. The C-terminus also is the region of the protein whose amino acid sequence shares specific amino acid sequence homology with

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particular proteins in the TGF- β super-family of regulatory proteins, and which includes the conserved cysteine skeleton.

Approximately 7×10^5 phages of an oligo(dT) primed
05 17.5 days p.c. mouse embryo 5' stretch cDNA (gt10)
library (Clonetech, Inc., Palo Alto, CA) was screened
with the labelled probe. The screen was performed
using the following stringent hybridization conditions:
40% formamide, 5 x SSPE, 5 x Denhart's solution, 0.1%
10 SDS, at 37°C overnight, and washing in 0.1 x SSPE, 0.1%
SDS at 50°C.

Five recombinant phages were purified over three rounds of screening. Phage DNA was prepared from all five phages, subjected to an EcoRI digest, subcloned
15 into the EcoRI site of a common pUC-type plasmid modified to allow single strand sequencing, and sequenced using means well known in the art.

Two different DNAs were identified by this procedure. One DNA, referred to herein as mOP1, has
20 substantial homology to the mature form of OP1 (about 98%), and is described in detail in copending USSN 600,024, filed 18-Oct-90. A second DNA, encoding the C-terminus of a related gene and referred to herein as mOP2, also was identified by this procedure. The
25 N-terminus of the gene encoding mOP2 was identified subsequently by screening a second mouse cDNA library (Mouse PCC4 cDNA (ZAP) library, Stratagene, Inc., La Jolla, CA).

Mouse OP2 (mOP2) protein shares significant amino
30 acid sequence homology with the amino acid sequence of the hOP1 active region, e.g., OPS or OP7, about 74%

homology, and less homology with the intact mature form, e.g., OP1-18, about 58% homology. The cDNA sequence, and the encoded amino acid sequence, for the full length mOP-2 protein is depicted in Sequence ID 05 No. 3. The full-length form of the protein is referred to as the prepro form of mOP-2 ("mOP2-PP"), and includes a signal peptide sequence at its N-terminus. The amino acid sequence Leu-Ala-Leu-Cys-Ala-Leu (amino acid residues 13-18 of Sequence ID No. 3) is believed 10 to constitute the cleavage site for the removal of the signal peptide sequence, leaving an intermediate form of the protein, the "pro" form, to be secreted from the expressing cell. The amino acid sequence Arg-Ala-Pro-Arg-Ala (amino acid residues 255-259 of Sequence ID 15 No. 3) is believed to constitute the cleavage site that produces the mature form of the protein, herein referred to as "mOP2-A", and described by residues 259-397 of Seq. ID No. 3. Residues 301-397 of Seq. ID No. 3 correspond to the region defining the conserved 20 six cysteine skeleton. Residues 296-397 of Seq. ID No. 3 correspond to the region defining the conserved seven cysteine skeleton.

Using a probe prepared from the pro region of mOP2 (an EcoR1-BamH1 digest fragment, bp 467-771 of Sequence 25 ID No. 3), a human hippocampus library was screened (human hippocampus cDNA lambda (ZAP II library Stratagene, Inc., La Jolla, CA) following essentially the same procedure as for the mouse library screens. The procedure identified the N-terminus of a novel DNA 30 encoding an amino acid sequence having substantial homology with mOP2. The C-terminus of the gene subsequently was identified by probing a human genomic library (in lambda phage EMBL-3, Clonetech, Inc., Palo Alto, CA) with a labelled fragment from the novel human

DNA in hand. The novel polypeptide chain encoded by this DNA is referred to herein as hOP2 protein, and shares almost complete amino acid identity (about 92% amino acid sequence homology) with mOP2-A (see Fig. 1
05 and infra).

The cDNA sequence, and the encoded amino acid sequence, for the prepro form of hOP2, "hOP2-PP", is described in Sequence ID No. 5. This full-length form of the protein also includes a signal peptide sequence at its N-terminus. The amino acid sequence Leu-Ala-Leu-Cys-Ala-Leu (amino acid residues 13-18 of Sequence ID No. 5) is believed to constitute the cleavage site for the removal of the signal peptide sequence, leaving an intermediate form of the protein, the "pro" form, to be secreted from the expressing cell. The amino acid sequence Arg-Thr-Pro-Arg-Ala (amino acid residues 257-261 of Sequence ID No. 5) is believed to constitute the cleavage site that produces what is believed to be the mature form of the protein, herein referred to as
10 hOP2-A" and described by residues 261-399 of Seq. ID
15 No. 5.

Additional mature species of hOP2 thought to be active include truncated sequences, "hOP2-P" (described by residues 264-399 of Seq. ID No. 5) and "hOP2-R" (described by residues 267-399 of Seq. ID No. 5), and a slightly longer sequence ("hOP2-S", described by residues 240-399 of Seq. ID No. 5). Residues 303-399 of Seq. ID No. 5 correspond to the region defining the conserved six cysteine skeleton. Residues 297-399 of
20 Seq. ID No. 5 correspond to the region defining the
25 conserved seven cystein skeleton.
30

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It should be noted that the nucleic acid sequence encoding the N-terminus of the prepro form of both mOP2 and hOP2 is rich in guanidine and cytosine base pairs. As will be appreciated by those skilled in the art, sequencing such a "G-C rich" region can be problematic, due to stutter and/or band compression. Accordingly, the possibility of sequencing errors in this region can not be ruled out. However, the definitive amino acid sequence for these and other, similarly identified proteins can be determined readily by expressing the protein from recombinant DNA using, for example, any of the means disclosed herein, and sequencing the polypeptide chain by conventional peptide sequencing methods well known in the art.

Figure 1 compares the amino acid sequences of mature mOP2 and hOP2. Identity is indicated by three dots (...) in the mOP2 sequence. As is evident from the figure, the amino acid sequence homology between the mature forms of these two proteins is substantial (92% homology between the mature sequences, about 95% homology within the C-terminal active region (e.g., residues 38-139 or 42-139 of Fig. 1.)

Fig. 2 compares the amino acid sequences for the mature forms of all four species of OP1 and OP2 proteins. Here again, identity is indicated by three dots (...). Like the mOP2 protein, the hOP2 protein shares significant homology (about 74%) with the amino acid sequence defining the OP1 active region (OPS or OP7, residues 43-139 and 38-139, respectively, in Fig. 2), and less homology with OP1-18 (about 58% homology). Both OP2 proteins share the conserved seven cysteine skeleton seen in the OP1 proteins. In

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addition, the OP2 proteins comprise an eighth cysteine residue within this region (see position 78 in FIG. 2).

A preferred generic amino acid sequence useful
5 as a subunit of a dimeric osteogenic protein capable of inducing endochondral bone or cartilage formation when implanted in a mammal in association with a matrix, and which incorporates the maximum homology between the identified OP1 and OP2 proteins, can be described by
10 the sequence referred to herein as "OPX", described below and in Seq. No.7.

	Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe		
	1	5	10
15	Xaa Asp Leu Gly Trp Xaa Asp Trp Xaa Ile		
	15	20	
	Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys		
	25	30	
	Glu Gly Glu Cys Xaa Phe Pro Leu Xaa Ser		
20	35	40	
	Xaa Met Asn Ala Thr Asn His Ala Ile Xaa		
	45	50	
	Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa		
	55	60	
25	Xaa Val Pro Lys Xaa Cys Cys Ala Pro Thr		
	65	70	
	Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa		
	75	80	
	Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys		
30	85	90	
	Xaa Arg Asn Met Val Val Xaa Ala Cys Gly		
	95	100	
	Cys His,		

and wherein Xaa at res. 2 = (Lys or Arg); Xaa at res. 3 = (Lys or Arg); Xaa at res. 9 = (Ser or Arg); Xaa at res. 11 = (Arg or Gln); Xaa at res. 16 = (Gln or Leu); Xaa at res. 19 = (Ile or Val); Xaa at res. 23 = 5 (Glu or Gln); Xaa at res. 26 = (Ala or Ser); Xaa at res. 35 = (Ala or Ser); Xaa at res. 39 = (Asn or Asp); Xaa at res. 41 = (Tyr or Cys); Xaa at res. 50 = (Val or Leu); Xaa at res. 52 = (Ser or Thr); Xaa at res. 56 = (Phe or Leu); Xaa at res. 57 = (Ile or Met); Xaa at 10 res. 58 = (Asn or Lys); Xaa at res. 60 = (Glu, Asp or Asn); Xaa at res. 61 = (Thr, Ala or Val); Xaa at res. 65 = (Pro or Ala); Xaa at res. 71 = (Gln or Lys); Xaa at res. 73 = (Asn or Ser); Xaa at res. 75 = (Ile or Thr); Xaa at res. 80 = (Phe or Tyr); Xaa at res. 82 = 15 (Asp or Ser); Xaa at res. 84 = (Ser or Asn); Xaa at res. 87 = (Ile or Asp); Xaa at res. 89 = (Lys or Arg); Xaa at res. 91 = (Tyr, Ala or His); and Xaa at res. 97 = (Arg or Lys).

20 The high degree of homology exhibited between the various OP1 and OP2 proteins suggests that the novel osteogenic proteins identified herein will purify essentially as OP1 does, or with only minor modifications of the protocols disclosed for OP1.

25 Similarly, the purified mOP1, mOP2, and hOP2 proteins are predicted to have an apparent molecular weight of about 18 kDa as reduced single subunits, and an apparent molecular weight of about 36 kDa as oxidized dimers, as determined by comparison with molecular 30 weight standards on an SDS-polyacrylamide electrophoresis gel. Unglycosylated dimers (e.g., proteins produced by recombinant expression in E. coli) are predicted to have an apparent molecular weight of about 27 kDa. There appears to be one potential N

glycosylation site in the mature forms of the mOP2 and hOP2 proteins.

The identification of osteogenic proteins having an active region comprising eight cysteine residues also allows one to construct osteogenic polypeptide chains patterned after either of the following template amino acid sequences, or to identify additional osteogenic proteins having this sequence. The template sequences contemplated are "OPX-7C", comprising the conserved six cysteine skeleton plus the additional cysteine residue identified in the OP2 proteins, and "OPX-8C", comprising the conserved seven cysteine skeleton plus the additional cysteine residue identified in the OP2 proteins. The OPX-7C and OPX-8C sequences are described below and in Seq. ID Nos. 8 and 9, respectively. Each Xaa in these template sequences independently represents one of the 20 naturally-occurring L-isomer, α -amino acids, or a derivative thereof. Biosynthetic constructs patterned after this template readily are constructed using conventional DNA synthesis or peptide synthesis techniques well known in the art. Once constructed, osteogenic proteins comprising these polypeptide chains can be tested as disclosed herein.

25 "OPX-7C" (Sequence ID No. 8):

- 24 -

	35	40	
	Xaa		
	45	50	55
	Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa Xaa Xaa		
05		60	65
	Xaa		
	70	75	
	Xaa		
	80	85	
10	Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys Xaa		
	90	95	

"OPX-8C" (Sequence ID No. 9 comprising additional five residues at the N-terminus, including a conserved cysteine residue):

15	Cys Xaa		
	1	5	10
	Xaa		
	15	20	
	Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa		
20		25	30
	Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa		
	35	40	45
	Xaa		
	50	55	
25	Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys		
		60	65
	Cys Xaa		
	70	75	
	Xaa		
30		80	85
	Xaa Cys		
	90	95	
	Xaa Cys Xaa		

100

MATRIX PREPARATION

A. General Consideration of Matrix Properties

The currently preferred carrier material is a
05 xenogenic bone-derived particulate matrix treated as
disclosed herein. This carrier may be replaced by
either a biodegradable-synthetic or synthetic-inorganic
matrix (e.g., hydroxylapatite (HAP), collagen,
tricalcium phosphate or polylactic acid, polyglycolic
10 acid and various copolymers thereof.)

Studies have shown that surface charge, particle
size, the presence of mineral, and the methodology for
combining matrix and osteogenic protein all play a role
in achieving successful bone induction. Perturbation
15 of the charge by chemical modification abolishes the
inductive response. Particle size influences the
quantitative response of new bone; particles between
75 μm and 420 μm elicit the maximum response.
Contamination of the matrix with bone mineral will
20 inhibit bone formation. Most importantly, the
procedures used to formulate OP onto the matrix are
extremely sensitive to the physical and chemical state
of both the osteogenic protein and the matrix.

The sequential cellular reactions in the
25 interface of the bone matrix/osteogenic protein
implants are complex. The multistep cascade includes:
binding of fibrin and fibronectin to implanted matrix,
chemotaxis of cells, proliferation of fibroblasts,
differentiation into chondroblasts, cartilage

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formation, vascular invasion, bone formation, remodeling, and bone marrow differentiation.

A successful carrier for osteogenic protein must perform several important functions. It must bind
05 osteogenic protein and act as a slow release delivery system, accommodate each step of the cellular response during bone development, and protect the osteogenic protein from nonspecific proteolysis. In addition, selected materials must be biocompatible in vivo and
10 preferably biodegradable; the carrier must act as a temporary scaffold until replaced completely by new bone. Polylactic acid (PLA), polyglycolic acid (PGA), and various combinations have different dissolution rates in vivo. In bones, the dissolution rates can
15 vary according to whether the implant is placed in cortical or trabecular bone.

Matrix geometry, particle size, the presence of surface charge, and the degree of both intra-and-inter-particle porosity are all important to successful
20 matrix performance. It is preferred to shape the matrix to the desired form of the new bone and to have dimensions which span non-union defects. Rat studies show that the new bone is formed essentially having the dimensions of the device implanted.

The matrix may comprise a shape-retaining
25 solid made of loosely adhered particulate material, e.g., with collagen. It may also comprise a molded, porous solid, or simply an aggregation of close-packed particles held in place by surrounding tissue.
30 Masticated muscle or other tissue may also be used. Large allogenic bone implants can act as a carrier for the matrix if their marrow cavities are cleaned and

packed with particle and the dispersed osteogenic protein.

The preferred matrix material, prepared from xenogenic bone and treated as disclosed herein, 05 produces an implantable material useful in a variety of clinical settings. In addition to its use as a matrix for bone formation in various orthopedic, periodontal, and reconstructive procedures, the matrix also may be used as a sustained release carrier, or as a 10 collagenous coating for implants. The matrix may be shaped as desired in anticipation of surgery or shaped by the physician or technician during surgery. Thus, the material may be used for topical, subcutaneous, intraperitoneal, or intramuscular implants; it may be 15 shaped to span a nonunion fracture or to fill a bone defect. In bone formation or conduction procedures, the material is slowly absorbed by the body and is replaced by bone in the shape of or very nearly the shape of the implant.

20 Various growth factors, hormones, enzymes, therapeutic compositions, antibiotics, and other body treating agents also may be absorbed onto the carrier material and will be released over time when implanted as the matrix material is slowly absorbed. Thus, 25 various known growth factors such as EGF, PDGF, IGF, FGF, TGF- α , and TGF- β may be released in vivo. The material can be used to release chemotherapeutic agents, insulin, enzymes, or enzyme inhibitors.

B. Bone-Derived Matrices

1. Preparation of Demineralized Bone

Demineralized bone matrix, preferably bovine bone matrix, is prepared by previously published procedures (Sampath and Reddi (1983) Proc. Natl. Acad. Sci. USA 80:6591-6595). Bovine diaphyseal bones (age 05 1-10 days) are obtained from a local slaughterhouse and used fresh. The bones are stripped of muscle and fat, cleaned of periosteum, demarrowed by pressure with cold water, dipped in cold absolute ethanol, and stored at -20°C. They are then dried and fragmented by crushing 10 and pulverized in a large mill. Care is taken to prevent heating by using liquid nitrogen. The pulverized bone is milled to a particle size in the range of 70-850 μm , preferably 150-420 μm , and is defatted by two washes of approximately two hours 15 duration with three volumes of chloroform and methanol (3:1). The particulate bone is then washed with one volume of absolute ethanol and dried over one volume of anhydrous ether yielding defatted bone powder. The defatted bone powder is then demineralized by four 20 successive treatments with 10 volumes of 0.5 N HCl at 4°C for 40 min. Finally, neutralizing washes are done on the demineralized bone powder with a large volume of water.

2. Guanidine Extraction

25 Demineralized bone matrix thus prepared is extracted with 5 volumes of 4 M guanidine-HCl, 50mM Tris-HCl, pH 7.0 for 16 hr. at 4°C. The suspension is filtered. The insoluble material is collected and used to fabricate the matrix. The material is mostly 30 collagenous in nature. It is devoid of osteogenic or chondrogenic activity.

3. Matrix Treatments

The major component of all bone matrices is Type-I collagen. In addition to collagen, demineralized bone extracted as disclosed above 05 includes non-collagenous proteins which may account for 5% of its mass. In a xenogenic matrix, these noncollagenous components may present themselves as potent antigens, and may constitute immunogenic and/or inhibitory components. These components also may 10 inhibit osteogenesis in allogenic implants by interfering with the developmental cascade of bone differentiation. It has been discovered that treatment of the matrix particles with a collagen fibril-modifying agent extracts potentially unwanted 15 components from the matrix, and alters the surface structure of the matrix material. Useful agents include acids, organic solvents or heated aqueous media. Various treatments are described below. A detailed physical analysis of the effect these fibril-modifying agents have on demineralized, quanidine-extracted bone collagen particles is disclosed in PCT 20 WO 90/10018, published 7-SEP-90.

After contact with the fibril-modifying agent, the treated matrix is washed to remove any extracted 25 components, following a form of the procedure set forth below:

1. Suspend in TBS (Tris-buffered saline) 1g/200 ml and stir at 4°C for 2 hrs; or in 6 M urea, 50 mM Tris-HCl, 500 mM NaCl, pH 7.0 (UTBS) or water and 30 stir at room temperature (RT) for 30 minutes (sufficient time to neutralize the pH);

- 30 -

2. Centrifuge and repeat wash step; and
3. Centrifuge; discard supernatant; water wash residue; and then lyophilize.

3.1 Acid Treatments

- 05 1. Trifluoroacetic acid.

Trifluoroacetic acid is a strong non-oxidizing acid that is a known swelling agent for proteins, and which modifies collagen fibrils.

10 Bovine bone residue prepared as described above is sieved, and particles of the appropriate size are collected. These particles are extracted with various percentages (1.0% to 100%) of trifluoroacetic acid and water (v/v) at 0°C or room temperature for 1-2 hours with constant stirring. The treated matrix is 15 filtered, lyophilized, or washed with water/salt and then lyophilized.

2. Hydrogen Fluoride.

Like trifluoroacetic acid, hydrogen fluoride is a strong acid and swelling agent, and also is 20 capable of altering intraparticle surface structure. Hydrogen fluoride is also a known deglycosylating agent. As such, HF may function to increase the osteogenic activity of these matrices by removing the antigenic carbohydrate content of any glycoproteins 25 still associated with the matrix after guanidine extraction.

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Bovine bone residue prepared as described above is sieved, and particles of the appropriate size are collected. The sample is dried in vacuo over P_2O_5 , transferred to the reaction vessel and exposed to
05 anhydrous hydrogen fluoride (10-20 ml/g of matrix) by distillation onto the sample at $-70^{\circ}C$. The vessel is allowed to warm to $0^{\circ}C$ and the reaction mixture is stirred at this temperature for 120 minutes. After evaporation of the hydrogen fluoride in vacuo, the
10 residue is dried thoroughly in vacuo over KOH pellets to remove any remaining traces of acid. Extent of deglycosylation can be determined from carbohydrate analysis of matrix samples taken before and after treatment with hydrogen fluoride, after washing the
15 samples appropriately to remove non-covalently bound carbohydrates. SDS-extracted protein from HF-treated material is negative for carbohydrate as determined by Con A blotting.

The deglycosylated bone matrix is next washed
20 twice in TBS (Tris-buffered saline) or UTBS, water-washed, and then lyophilized.

Other acid treatments are envisioned in addition to HF and TFA. TFA is a currently preferred acidifying reagent in these treatments because of its
25 volatility. However, it is understood that other, potentially less caustic acids may be used, such as acetic or formic acid.

3.2 Solvent Treatment

1. Dichloromethane.

Dichloromethane (DCM) is an organic solvent capable of denaturing proteins without affecting their primary structure. This swelling agent is a common reagent in automated peptide synthesis, and is used in 05 washing steps to remove components.

Bovine bone residue, prepared as described above, is sieved, and particles of the appropriate size are incubated in 100% DCM or, preferably, 99.9% DCM/0.1% TFA. The matrix is incubated with the 10 swelling agent for one or two hours at 0°C or at room temperature. Alternatively, the matrix is treated with the agent at least three times with short washes (20 minutes each) with no incubation.

2. Acetonitrile.

15 Acetonitrile (ACN) is an organic solvent, capable of denaturing proteins without affecting their primary structure. It is a common reagent used in high-performance liquid chromatography, and is used to elute proteins from silica-based columns by perturbing 20 hydrophobic interactions.

Bovine bone residue particles of the appropriate size, prepared as described above, are treated with 100% ACN (1.0 g/30 ml) or, preferably, 99.9% ACN/0.1% TFA at room temperature for 1-2 hours 25 with constant stirring. The treated matrix is then water-washed, or washed with urea buffer, or 4 M NaCl and lyophilized. Alternatively, the ACN or ACN/TFA treated matrix may be lyophilized without wash.

3. Isopropanol.

Isopropanol is also an organic solvent capable of denaturing proteins without affecting their primary structure. It is a common reagent used to elute proteins from silica HPLC columns.

05 Bovine bone residue particles of the appropriate size prepared as described above are treated with 100% isopropanol (1.0 g/30 ml) or, preferably, in the presence of 0.1% TFA, at room temperature for 1-2 hours with constant stirring. The
10 matrix is then water-washed or washed with urea buffer or 4 M NaCl before being lyophilized.

4. Chloroform

Chloroform also may be used to increase surface area of bone matrix like the reagents set forth
15 above, either alone or acidified.

Treatment as set forth above is effective to assure that the material is free of pathogens prior to implantation.

3.3 Heat Treatment

20 The currently most preferred agent is a heated aqueous fibril-modifying medium such as water, to increase the matrix particle surface area and porosity. The currently most preferred aqueous medium is an acidic aqueous medium having a pH of less than about
25 4.5, e.g., within the range of pH 2 - pH 4. which may help to "swell" the collagen before heating. 0.1% acetic acid, which has a pH of about 3, currently is preferred. 0.1 M acetic acid also may be used.

Various amounts of delipidated, demineralized guanidine-extracted bone collagen are heated in the aqueous medium (1g matrix/30ml aqueous medium) under constant stirring in a water jacketed glass flask, and
05 maintained at a given temperature for a predetermined period of time. Preferred treatment times are about one hour, although exposure times of between about 0.5 to two hours appear acceptable. The temperature employed is held constant at a temperature generally
10 within the range of about 37°C to 75°C. The currently preferred heat treatment temperature is within the range of 45°C to 60°C.

After the heat treatment, the matrix is filtered, washed, lyophilized and used for implant. Where an
15 acidic aqueous medium is used, the matrix also is preferably neutralized prior to washing and lyophilization. A currently preferred neutralization buffer is a 200mM sodium phosphate buffer, pH 7.0. To neutralize the matrix, the matrix preferably first is
20 allowed to cool following thermal treatment, the acidic aqueous medium (e.g., 0.1% acetic acid) then is removed and replaced with the neutralization buffer and the matrix agitated for about 30 minutes. The neutralization buffer then may be removed and the
25 matrix washed and lyophilized (see infra).

The matrix also may be treated to remove contaminating heavy metals, such as by exposing the matrix to a metal ion chelator. For example, following thermal treatment with 0.1% acetic acid, the matrix may
30 be neutralized in a neutralization buffer containing EDTA (sodium ethylenediaminetetraacetic acid), e.g., 200 mM sodium phosphate, 5mM EDTA, pH 7.0. 5 mM EDTA provides about a 100-fold molar excess of chelator to

residual heavy metals present in the most contaminated matrix tested to date. Subsequent washing of the matrix following neutralization appears to remove the bulk of the EDTA. EDTA treatment of matrix particles 05 reduces the residual heavy metal content of all metals tested (Sb, As, Be, Cd, Cr, Cu, Co, Pb, Hg, Ni, Se, Ag, Zn, Tl) to less than about 1 ppm. Bioassays with EDTA-treated matrices indicate that treatment with the metal ion chelator does not inhibit bone inducing activity.

10 The collagen matrix materials preferably take the form of a fine powder, insoluble in water, comprising nonadherent particles. It may be used simply by packing into the volume where new bone growth or sustained release is desired, held in place by 15 surrounding tissue. Alternatively, the powder may be encapsulated in, e.g., a gelatin or polylactic acid coating, which is adsorbed readily by the body. The powder may be shaped to a volume of given dimensions and held in that shape by interadhering the particles 20 using, for example, soluble, species-biocompatible collagen. The material may also be produced in sheet, rod, bead, or other macroscopic shapes.

Demineralized rat bone matrix used as an allogenic matrix in certain of the experiments 25 disclosed herein, is prepared from several of the dehydrated diaphyseal shafts of rat femur and tibia as described herein to produce a bone particle size which passes through a 420 μm sieve. The bone particles are subjected to dissociative extraction with 4 M 30 guanidine-HCl. Such treatment results in a complete loss of the inherent ability of the bone matrix to induce endochondral bone differentiation. The remaining insoluble material is used to fabricate the

matrix. The material is mostly collagenous in nature, and upon implantation, does not induce cartilage and bone. All new preparations are tested for mineral content and osteogenic activity before use. The total
05 loss of biological activity of bone matrix is restored when an active osteoinductive protein fraction or a pure osteoinductive protein preparation is reconstituted with the biologically inactive insoluble collagenous matrix.

10

FABRICATION OF OSTEOGENIC DEVICE

The naturally sourced and recombinant protein as set forth above, and other constructs, can be combined and dispersed in a suitable matrix preparation using any of the methods described below. In general, 50-100
15 ng of active protein is combined with the inactive carrier matrix (e.g., 25 mg for rat bioassays). Greater amounts may be used for large implants.

1. Ethanol Precipitation

Matrix is added to osteogenic protein
20 dissolved in guanidine-HCl. Samples are vortexed and incubated at a low temperature (e.g., 4°C). Samples are then further vortexed. Cold absolute ethanol (5 volumes) is added to the mixture which is then stirred and incubated, preferably for 30 minutes at -20°C.
25 After centrifugation (microfuge, high speed) the supernatant is discarded. The reconstituted matrix is washed twice with cold concentrated ethanol in water (85% EtOH) and then lyophilized.

2. Acetonitrile Trifluoroacetic

Acid Lyophilization

In this procedure, osteogenic protein in an acetonitrile trifluoroacetic acid (ACN/TFA) solution is added to the carrier material. Samples are vigorously 05 vortexed many times and then lyophilized. This method is currently preferred, and has been tested with osteogenic protein at varying concentrations and different levels of purity.

3. Urea Lyophilization

10 For those osteogenic proteins that are prepared in urea buffer, the protein is mixed with the matrix material, vortexed many times, and then lyophilized. The lyophilized material may be used "as is" for implants.

15 4. Buffered Saline Lyophilization

OP1 and OP2 preparations in physiological saline may also be vortexed with the matrix and lyophilized to produce osteogenically active material.

20 These procedures also can be used to adsorb other active therapeutic drugs, hormones, and various bioactive species to the matrix for sustained release purposes.

BIOASSAY

The functioning of the various proteins and 25 devices of this invention can be evaluated with an in vivo bioassay. Studies in rats show the osteogenic effect in an appropriate matrix to be dependent on the

dose of osteogenic protein dispersed in the matrix. No activity is observed if the matrix is implanted alone. In vivo bioassays performed in the rat model also have shown that demineralized, guanidine-extracted xenogenic bone matrix materials of the type described in the literature are ineffective as a carrier, fail to induce bone, and produce an inflammatory and immunological response when implanted unless treated as disclosed above. In certain species (e.g., monkey) allogenic matrix materials also apparently are ineffective as carriers. The following sets forth various procedures for preparing osteogenic devices from the proteins and matrix materials prepared as set forth above, and for evaluating their osteogenic utility.

15 A. Rat Model

1. Implantation

The bioassay for bone induction as described by Sampath and Reddi ((1983) Proc. Natl. Acad. Sci. USA 80 6591-6595), herein incorporated by reference, may be used to monitor endochondral bone differentiation activity. This assay consists of implanting test samples in subcutaneous sites in recipient rats under ether anesthesia. Male Long-Evans rats, aged 28-32 days, were used. A vertical incision (1 cm) is made under sterile conditions in the skin over the thoracic region, and a pocket is prepared by blunt dissection. Approximately 25 mg of the test sample is implanted deep into the pocket and the incision is closed with a metallic skin clip. The day of implantation is designated as day one of the experiment. Implants were removed on day 12. The heterotopic site allows for the study of bone induction without the possible

ambiguities resulting from the use of orthotropic sites. As disclosed herein, both allogenic (rat bone matrix) and xenogenic (bovine bone matrix) implants were assayed.

05 2. Cellular Events

Successful implants exhibit a controlled progression through the stages of protein-induced endochondral bone development, including: (1) transient infiltration by polymorphonuclear leukocytes on day 10 one; (2) mesenchymal cell migration and proliferation on days two and three; (3) chondrocyte appearance on days five and six; (4) cartilage matrix formation on day seven; (5) cartilage calcification on day eight; (6) vascular invasion, appearance of osteoblasts, and formation of new bone on days nine and ten; (7) appearance of osteoblastic and bone remodeling and dissolution of the implanted matrix on days twelve to eighteen; and (8) hematopoietic bone marrow differentiation in the ossicle on day twenty-one. The 20 results show that the shape of the new bone conforms to the shape of the implanted matrix.

3. Histological Evaluation

Histological sectioning and staining is preferred to determine the extent of osteogenesis in 25 the implants. Implants are fixed in Bouins Solution, embedded in paraffin, and cut into 6-8 μm sections. Staining with toluidine blue or hematoxylin/eosin demonstrates clearly the ultimate development of endochondral bone. Twelve day implants are usually 30 sufficient to determine whether the implants contain newly induced bone.

4. Biological Markers

Alkaline phosphatase activity may be used as a marker for osteogenesis. The enzyme activity may be determined spectrophotometrically after homogenization 05 of the implant. The activity peaks at 9-10 days in vivo and thereafter slowly declines. Implants showing no bone development by histology have little or no alkaline phosphatase activity under these assay conditions. The assay is useful for quantitation and 10 obtaining an estimate of bone formation quickly after the implants are removed from the rat. Alternatively, the amount of bone formation can be determined by measuring the calcium content of the implant.

The invention may be embodied in other specific 15 forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather 20 than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 05 (i) APPLICANT: OPPERMANN, HERMANN
OZKAYNAK, ENGIN
KUBERASAMPATH, THANGAVEL
RUEGER, DAVID C.

(ii) TITLE OF INVENTION: OSTEOGENIC DEVICES

(iii) NUMBER OF SEQUENCES: 9

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15 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

20 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

25 (viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 27,829
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(B) TELEFAX: 617/248-7100

(2) INFORMATION FOR SEQ ID NO:1:

- 42 -

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: HIPPOCAMPUS

05 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 49..1341
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "hOP1-PP"
 /evidence= EXPERIMENTAL
 /standard_name= "hOP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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	10	
	15	
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	25	
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	95	
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	105	
	110	
	115	
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	Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp	

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	200	205	210	
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30	TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile			921
	280	285	290	
	CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro			969
	295	300	305	
35	AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser			1017
	310	315	320	
	AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe			1065
40	325	330	335	

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05	CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala 340 345 350 355	1113
10	GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met 360 365 370	1161
	AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn 375 380 385	1209
15	CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala 390 395 400	1257
	ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys 405 410 415	1305
20	TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His 420 425 430	1351
	GAGAATTCAAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG	1411
	GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCCTC CCTATCCCCA ACTTTAAAGG	1471
25	TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTG ATCAGTTTT CAGTGGCAGC ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC GCATAAAAGAA AAATGCCGG GCCAGGTCA TGGCTGGAA GTCTCAGCCA TGCACGGACT CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCAAAG GAAAATTGAC CCGGAAGTTC	1531 1591 1651 1711 1771
30	CTGTAATAAA TGTACAATA AAACGAATGA ATGAAAAAAA AAAAAAAA A	1822

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 431 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:

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05

(D) OTHER INFORMATION: /Product="hOP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala				
1	5	10	15		
10	Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser	20	25	30	
	Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser	35	40	45	
	Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu	50	55	60	
15	Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro	65	70	75	80
	Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly	85	90	95	
20	Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser	100	105	110	
	Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr	115	120	125	
	Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys	130	135	140	
25	Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu	145	150	155	160
	Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile	165	170	175	
30	Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile	180	185	190	
	Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu	195	200	205	
	Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu	210	215	220	
	Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg	225	230	235	240
	His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser	245	250	255	
	Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn				

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05	260	265	270
	Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe		
	275	280	285
	Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser		
	290	295	300
10	Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu		
	305	310	315
	Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr		
	325	330	335
15	Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu		
	340	345	350
	Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn		
	355	360	365
	Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His		
	370	375	380
20	Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln		
	385	390	395
	Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile		
	405	410	415
25	Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His		
	420	425	430

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1929 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 103..1293
 - (D) OTHER INFORMATION: /function= "osteogenic protein"
 /product= "mOP2-PP"
 /note= "mOP2 cDNA"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCCGCT GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC

60

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05	CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT Met Ala Met Arg 1	114
10	CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly 5 10 15 20	162
	Gly His Gly Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly 25 30 35	210
15	GCG CGC GAC CGG GAC ATG CAG CGT GAA ATC CTG CCG GTG CTC GGG CTA Ala Arg Asp Arg Asp Met Gln Arg Glu Ile Leu Pro Val Leu Gly Leu 40 45 50	258
	CCG GGA CGC CCC GAC CCC GTG CAC AAC CCG CCG CTG CCC GGC ACG CAG Pro Gly Arg Pro Asp Pro Val His Asn Pro Pro Leu Pro Gly Thr Gln 55 60 65	306
20	CGT GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC Arg Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp 70 75 80	354
25	GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val 85 90 95 100	402
	ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln 105 110 115	450
30	GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala 120 125 130	498
	GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA CCC AGC Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser 135 140 145	546
	ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val 150 155 160	594
	CAA GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln 165 170 175 180	642
	ACG CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala 185 190 195	690
	GCC AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC	738

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05	Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg 200 205 210	
	CTC TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala 215 220 225	786
10	GGT CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val 230 235 240	834
15	ACC TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala 245 250 255 260	882
	AGA CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His 265 270 275	930
20	CCC AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly 280 285 290	978
	AGA GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGA TTC CGT GAC CTT Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Arg Phe Arg Asp Leu 295 300 305	1026
25	GCG TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr 310 315 320	1074
30	TGT GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr 325 330 335 340	1122
	AAC CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val 345 350 355	1170
35	GTC CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val 360 365 370	1218
	CTG TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn 375 380 385	1266
	ATG GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC Met Val Val Lys Ala Cys Gly Cys His 390 395	1313
	TGCTTCTACT ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT	1373

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05	TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCAC TTCCCCTGGCCA CTTCCCTGCTA AAATTCTGGT CTTTCCCA GT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCCGCC CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCCAC AATGGCAAAT TCTGGATGGT CTAAGAACCCCTA CTGGAATTCT AAACTAGATG	1433 1493 1553 1613 1673
10	ATCTGGGCTC TCTGCACCAT TCATTGTGGC AGTTGGACA TTTTTAGGTA TAACAGACAC ATACACTTAG ATCAATGCAT CGCTGTACTC CTTGAAATCA GAGCTAGCTT GTTAGAAAAAA GAATCAGAGC CAGGTATAGC GGTGCATGTC ATTAATCCCA GCGCTAAAGA GACAGAGACA GGAGAATCTC TGTGAGTTCA AGGCCACATA GAAAGAGCCT GTCTCGGGAG CAGGAAAAAA	1733 1793 1853 1913
	AAAAAAAAACG GAATT	1929

15 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 397 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(ix) FEATURE:
 (D) OTHER INFORMATION: /Product= "mOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

25	Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys 1 5 10 15
	Ala Leu Gly Gly Gly His Gly Pro Gly Pro Pro His Thr Cys Pro Gln 20 25 30
	Arg Arg Leu Gly Ala Arg Asp Arg Asp Met Gln Arg Glu Ile Leu Pro 35 40 45
30	Val Leu Gly Leu Pro Gly Arg Pro Asp Pro Val His Asn Pro Pro Leu 50 55 60
	Pro Gly Thr Gln Arg Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala 65 70 75 80
	Met Thr Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg 85 90 95

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05	Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr			
	100	105	110	
	Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr			
	115	120	125	
10	Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr			
	130	135	140	
	Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met			
	145	150	155	160
	Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe			
	165	170	175	
15	Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu			
	180	185	190	
	Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp			
	195	200	205	
20	Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp			
	210	215	220	
	Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln			
	225	230	235	240
	Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala			
	245	250	255	
25	Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn			
	260	265	270	
	Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His			
	275	280	285	
30	Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Arg			
	290	295	300	
	Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr			
	305	310	315	320
	Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys			
	325	330	335	
35	Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met			
	340	345	350	
	Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser			
	355	360	365	
	Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg			
	370	375	380	

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05 Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
 385 390 395

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1941 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 15 (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
 - (F) TISSUE TYPE: HIPPOCAMPUS
- 20 (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 507..1703
 - (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "hOP2-PP"
 /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

25	GGAATTCCGG CCACAGTGGC GCCGGCAGAG CAGGAGTGGC TGGAGGAGCT GTGGTTGGAG	60
	CAGGAGGTGG CACGGCAGGG CTGGAGGGCT CCCTATGAGT GGCGGAGACG GCCCAGGAGG	120
	CGCTGGAGCA ACAGCTCCCA CACCGCACCA AGCGGTGGCT GCAGGAGCTC GCCCATCGCC	180
	CCTGCGCTGC TCGGACCGCG GCCACAGCCG GACTGGCGGG TACGGCGGCG ACAGAGGCAT	240
	TGGCCGAGAG TCCCAGTCCG CAGAGTAGGCC CCGGCCTCGA GGCGGTGGCG TCCCGGTCCCT	300
30	CTCCGTCCAG GAGCCAGGAC AGGTGTCGCG CGGCGGGGCT CCAGGGACCG CGCCTGAGGC	360
	CGGCTGCCCG CCCGTCCCCGC CCCGCCCGCG CGCCCGAGCC CAGCCTCCCT	420
	GGCGTGGGG CGTCCCCAGG CCCTGGGTCG GCCGCAGGAGC CGATGCGCGC CCGCTGAGCG	480
	CCCCAGCTGA GCGCCCCCGG CCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG	533
35	Met Thr Ala Leu Pro Gly Pro Leu Trp	
	1 5	
	CTC CTG GGC CTG GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG	581
	Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly Pro Gly Leu	
10	15 20 25	
	CGA CCC CCG CCC GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAC CGG	629

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05	Arg Pro Pro Pro Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Asp Arg		
	30	35	40
	GAC GTG CAG CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC		677
	Asp Val Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro		
	45	50	55
10	CGG CCC CGC GCG CCA CCC GCC TCC CGG CTG CCC GCG TCC GCG CCG		725
	Arg Pro Arg Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro		
	60	65	70
	CTC TTC ATG CTG GAC CTG TAC CAC CGC ATG GCC GGC GAC GAC GAC GAG		773
	Leu Phe Met Leu Asp Leu Tyr His Arg Met Ala Gly Asp Asp Asp Glu		
15	75	80	85
	GAC GGC GCC GCG GAG GCC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC		821
	Asp Gly Ala Ala Glu Ala Leu Gly Arg Ala Asp Leu Val Met Ser Phe		
	90	95	100
	105		
20	GTT AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT		869
	Val Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His		
	110	115	120
	TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG		917
	Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala		
	125	130	135
25	GTC ACA GCT GCG GAG TTC CCG ATT TAC AAG GTG CCC AGC ATC CAC CTG		965
	Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu		
	140	145	150
	CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG		1013
	Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln		
30	155	160	165
	TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA		1061
	Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg		
	170	175	180
	185		
35	GCT GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC		1109
	Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp		
	190	195	200
	TGC TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG		1157
	Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val		
	205	210	215
40	GAG ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG		1205
	Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu		
	220	225	230
	GGT CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC		1253
	Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe		

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	05	235	240	245		
	AGG	GCC	AGT	CCG	AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG	1301
	Arg	Ala	Ser	Pro	Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu	
	250		255		260	265
10	AGG	AGG	AGG	CAG	CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA	1349
	Arg	Arg	Arg	Gln	Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg	
	270		275		280	
	CTC	CCA	GGG	ATC	TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC	1397
	Leu	Pro	Gly	Ile	Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val	
	285		290		295	
15	TGC	CGT	CGG	CAC	GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG	1445
	Cys	Arg	Arg	His	Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu	
	300		305		310	
	GAC	TGG	GTC	ATC	GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG	1493
	Asp	Trp	Val	Ile	Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly	
20	315		320		325	
	GAG	TGC	TCC	TTC	CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC	1541
	Glu	Cys	Ser	Phe	Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala	
	330		335		340	345
25	ATC	CTG	CAG	TCC	CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG	1589
	Ile	Leu	Gln	Ser	Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys	
	350		355		360	
	GCG	TGC	TGT	GCA	CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT	1637
	Ala	Cys	Cys	Ala	Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr	
	365		370		375	
30	GAC	AGC	AGC	AAC	AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC	1685
	Asp	Ser	Ser	Asn	Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val	
	380		385		390	
	AAG	GCC	TGC	GGC	TGC CAC TGAGTCAGCC CGCCCAGCCC TACTGCAGCA	1733
	Lys	Ala	Cys	Gly	Cys His	
35	395					
	ATTCACTGGC	CGTCGTTTA	CAACGTGTGA	CTGGGAAAAC	CCTGGCGTTA CCCAACTTAA	1793
	TCGCCTTGCA	GCACATCCCC	CTTCGCCAG	CTGGCTAATA	GCGAAGAGGC CCCGCACCGA	1853
	TCGCCCTTCC	CAACAGTTGC	GCCCCAGTGA	ATGGCGAATG	GCAAATTGTA AGCGTTAATA	1913
	TTTGTAA	ATT CGCGTTA	AATTTTTT			1941

(2) INFORMATION FOR SEQ ID NO:6:

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05	Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser	
	225	230
	235	240
	Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile	
	245	250
	255	
10	Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys	
	260	265
	270	
	Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp	
	275	280
	285	
	Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr	
	290	295
	300	
15	Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln	
	305	310
	315	320
	Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp	
	325	330
	335	
20	Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His	
	340	345
	350	
	Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys	
	355	360
	365	
	Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile	
	370	375
	380	
25	Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly Cys His	
	385	390
	395	

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..102
 - (D) OTHER INFORMATION: /label= OPX
 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
 AS DEFINED IN THE SPECIFICATION

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
 1 5 10 15

5 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
 20 25 30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
 35 40 45

10 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
 50 55 60

15 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
 65 70 75 80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
 85 90 95

20 Xaa Ala Cys Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:8:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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05 Xaa
 65 70 75 80

Xaa Cys Xaa Cys
 85 90 95

Xaa

10 (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..102
 - (D) OTHER INFORMATION: /label= PROTEIN

20 /note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
 ONE OF THE 20 NATURALLY-OCCURRING L-ISOMER A-AMINO
 ACIDS, OR A DERIVATIVE THEREOF."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

25 Cys Xaa
 1 5 10 15

Xaa Cys Xaa Xaa
 20 25 30

Xaa Cys Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa
 35 40 45

30 Xaa
 50 55 60

Xaa Cys Cys Xaa
 65 70 75 80

Xaa
 85 90 95

Xaa Xaa Cys Xaa Cys Xaa
 100

05 What is claimed is:

1. A polypeptide chain comprising an amino acid sequence described by residues 303-399 of Seq. ID No. 5.
- 10 2. The polypeptide chain of claim 1 comprising an amino acid sequence described by residues 297-399 of Seq. ID No. 5.
3. The polypeptide chain of claim 2 comprising of amino acid sequence described by residues 267-399 of Seq. ID No. 5.
- 15 4. The polypeptide chain of claim 3 comprising an amino acid sequence described by residues 264-399 of Seq. ID No. 5.
- 20 5. The polypeptide chain of claim 4 comprising an amino acid sequence described by residues 240-399 of Seq. ID No. 5.
6. The polypeptide chain of claim 5 comprising an amino acid sequence described by residues 1-399 of Seq. ID No. 5.
- 25 7. A polypeptide chain comprising an amino acid sequence described by residues of 301-397 of Seq. ID No. 3.
8. The polypeptide chain of claim 7 comprising an amino acid sequence described by residues 296-397 of Seq. ID No.3.

- 05 9. The polypeptide chain of claim 8 comprising an amino acid sequence described by residues 259-397 of Seq. ID No. 3.
- 10 10. The polypeptide chain of claim 9 comprising an amino acid described by residues 1-397 of Seq. ID No. 3.
- 15 11. A polypeptide chain useful as a subunit of a dimeric osteogenic protein comprising a pair of disulfide-bonded polypeptide chains, said polypeptide chain having an amino acid sequence described by residues 303-399 of Seq. ID No. 5, including allelic and species variants thereof, such that the dimeric osteogenic protein comprising said polypeptide chain has a conformation capable of inducing endochondral bone formation when implanted in a mammal in association with a matrix.
- 20 12. The polypeptide chain of claim 11 wherein said amino acid sequence comprises residues 261-399 of Seq. ID 5.
- 25 13. The polypeptide chain of claim 11 wherein the amino acid sequence comprises residues 301-397 of Seq. ID No. 3.
- 30 14. The polypeptide chain of claim 13 wherein said amino acid sequent comprises residues 259-397 of Seq. ID No. 3.
- 30 15. A dimeric osteogenic protein capable of inducing endochondral bone formation in a mammal when implanted in said mammal in association with a matrix;

- 05 said protein comprising a pair of disulfide-bonded polypeptide chains constituting a dimeric species, wherein each said polypeptide chain is the polypeptide chain of claim 11.
- 10 16. The polypeptide chain of claim 3 or 11 produced by expression of recombinant DNA in a host cell.
- 15 17. The polypeptide chain of claim 16 wherein said host cell is a procaryotic host cell.
- 20 18. The polypeptide chain of claim 16 wherein said host cell is a mammalian cell.
- 25 19. The polypeptide of claim 1, 3 or 11 that is glycosylated.
- 30 20. A nucleic acid encoding the polypeptide chain of claim 1, 3, or 11.
- 35 21. A dimeric protein comprising a pair of polypeptide chains expressed from a DNA sequence described by ID No. 3 or ID No. 5, including allelic and species variants thereof, such that, when said polypeptide chains are oxidized to produce a disulfide-bonded dimeric species, the dimeric species has a conformation that is capable of inducing endochondral bone or cartilage formation when disposed within a matrix and implanted in a mammal.

hOP2	Ala	Val	Arg	Pro	Leu	Arg	Arg	Arg
mOP2	...	Ala	Lys
					5			
hOP2	Gln	Pro	Lys	Lys	Ser	Asn	Glu	Leu
mOP2	Thr
		10				15		
hOP2	Pro	Gln	Ala	Asn	Arg	Leu	Pro	Gly
mOP2	...	His	Pro	...	Lys
		20						
hOP2	Ile	Phe	Asp	Asp	Val	His	Gly	Ser
mOP2	Gly
	25					30		
hOP2	His	Gly	Arg	Gln	Val	Cys	Arg	Arg
mOP2	Arg	Glu	40
		35						
hOP2	His	Glu	Leu	Tyr	Val	Ser	Phe	Gln
mOP2	Arg	...	Arg
		45						
hOP2	Asp	Leu	Gly	Trp	Leu	Asp	Trp	Val
mOP2
		50					55	...
hOP2	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala
mOP2
		60						
hOP2	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ser
mOP2	Ala
		65				70		

Fig. 1.1

hOP2	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	
mOP2	
				75					80
hOP2	Ala	Thr	Asn	His	Ala	Ile	Leu	Gln	
mOP2	
						85			
hOP2	Ser	Leu	Val	His	Leu	Met	Lys	Pro	
mOP2	
		90					95		
hOP2	Asn	Ala	Val	Pro	Lys	Ala	Cys	Cys	
mOP2	Asp	Val	
				100					
hOP2	Ala	Pro	Thr	Lys	Leu	Ser	Ala	Thr	
mOP2	
	i05					i10			
hOP2	Ser	Val	Leu	Tyr	Tyr	Asp	Ser	Ser	
mOP2	
		i15					i20		
hOP2	Asn	Asn	Val	Ile	Leu	Arg	Lys	Ala	
mOP2	His	
					125				
hOP2	Arg	Asn	Met	Val	Val	Lys	Ala	Cys	
mOP2	
		i30				i35			
hOP2	Gly	Cys	His						
mOP2						

Fig. 1.2

hOP1	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln
mOP1	Gly
hOP2	Ala	Val	Arg	Pro	Leu	Arg	...	Arg	...
mOP2	Ala	Ala	Arg	Pro	Leu	Lys	...	Arg	...
	1					5			
hOP1	Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
mOP1
hOP2	Pro	Lys	Lys	Ser	Asn	Glü	Leu	Pro	Gln
mOP2	Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His
	10					15			
hOP1	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala
mOP1	Ser
hOP2	Ala	Asn	Arg	Leu	Pro	Gly	Ile	Phe	Asp
mOP2	Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp
	20					25			
hOP1	Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
mOP1
hOP2	Asp	Val	His	Gly	...	His	Gly
mOP2	Asp	Gly	His	Gly	...	Arg	Gly	...	Glu
	30					35			
hOP1	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
mOP1
hOP2	Val	...	Arg	Arg
mOP2	Val	...	Arg	Arg
	40					45			

Fig. 2.1

hOP1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP1
hOP2	Gln	Leu	...
mOP2	Arg	Leu	...
							10		
hOP1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
mOP1
hOP2	...	Val	Gln	Ser
mOP2	...	Val	Gln	Ser
	55					60			
hOP1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP1
hOP2	Ser
mOP2
	65					70			
hOP1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
mOP1
hOP2	Asp	...	Cys
mOP2	Asp	...	Cys
	75					80			
hOP1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
mOP1
hOP2	Leu	...	Ser	...
mOP2	Leu	...	Ser	...
	85					90			

Fig. 2.2

hOP1	Val His Phe Ile Asn Pro Glu Thr Val
mOP1 Asp
hOP2 Leu Met Lys ... Asn Ala ...
mOP2 Leu Met Lys ... Asp Val ...
	95
hOP1	Pro Lys Pro Cys Cys Ala Pro Thr Gln
mOP1
hOP2 Ala Lys
mOP2 Ala Lys
100	105
hOP1	Leu Asn Ala Ile Ser Val Leu Tyr Phe
mOP1
hOP2	... Ser ... Thr Tyr
mOP2	... Ser ... Thr Tyr
110	115
hOP1	Asp Asp Ser Ser Asn Val Ile Leu Lys
mOP1 Asp
hOP2	... Ser ... Asn Arg
mOP2	... Ser ... Asn Arg
120	125
hOP1	Lys Tyr Arg Asn Met Val Val Arg
mOP1
hOP2	... Ala Lys
mOP2	... His Lys
130	
hOP1	Ala Cys Gly Cys His
mOP1
hOP2
mOP2
135	

Fig. 2.3

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/07635

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.C1.5 A 61 K 37/02	C 12 N 15/00 A 61 K 27/00	C 07 K 7/10	C 07 K 13/00
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II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System	Classification Symbols	
Int.C1.5	C 07 K	A 61 K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched⁸III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	WO,A,8909788 (CREATIVE BIOMOLECULES) 19 October 1989, see the whole document	11,15- 21 1-10,12 -14
A	---	
X	WO,A,9011366 (GENETICS INSTITUTE) 4 October 1990, see the whole document	11,15- 21 11,15- 21
A	---	
X	EMBO Journal, volume 9, no. 7, 1990, Oxford University Press (Eynsham, Oxford, GB) E. Ozkaynak et al.: "OP-1 cDNA encodes an osteogenic protein in the TGF-beta family", pages 2085-2093, see the whole article -----	11,15- 21

¹⁰ Special categories of cited documents :

- ^{"A"} document defining the general state of the art which is not considered to be of particular relevance
- ^{"E"} earlier document but published on or after the international filing date
- ^{"L"} document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- ^{"O"} document referring to an oral disclosure, use, exhibition or other means
- ^{"P"} document published prior to the international filing date but later than the priority date claimed

^{"T"} later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention^{"X"} document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step^{"Y"} document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.^{"&"} document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

31-01-1992

Date of Mailing of this International Search Report

20 FEB 1992

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

MISS J. TAZELAAR

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

**US 9107635
SA 53017**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 12/02/92. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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WO-A- 8909788	19-10-89	US-A-	4968590	06-11-90
		US-A-	5011691	30-04-91
		AU-A-	3444989	03-11-89
		AU-A-	3530589	03-11-89
		EP-A-	0372031	13-06-90
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		JP-T-	3502579	13-06-91
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		EP-A-	0411105	06-02-91
		JP-T-	3504736	17-10-91
		WO-A-	9010018	07-09-90
		US-A-	4975526	04-12-90
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WO-A- 9011366	04-10-90	AU-A-	5357790	22-10-90
		CA-A-	2030518	29-09-90
		EP-A-	0429570	05-06-91
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